

PB85-198372

Sensitivity of Rainbow Trout
Early Life Stages to Nickel Chloride

(U.S.) Corvallis Environmental Research Lab., OR

1985

U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-85/016	2.	3. R PB85-198372
4. TITLE AND SUBTITLE Sensitivity of Rainbow Trout Early Life Stages to Nickel Chloride (Journal Version)	5. REPORT DATE 1985	
	6. PERFORMING ORGANIZATION CODE	
7. AUTHOR(S) Alan V. Nebeker, Carol Savonen, and Donald G. Stevens	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development 200 SW 35th Street Corvallis, Oregon 97333	10. PROGRAM ELEMENT NO.	
	11. CONTRACT/GRANT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Same	13. TYPE OF REPORT AND PERIOD COVERED Journal Article	
	14. SPONSORING AGENCY CODE EPA/600/02	
15. SUPPLEMENTARY NOTES Environmental Toxicology and Chemistry 4 (2): 233-239, 1985. April-June.		
16. ABSTRACT Four acute tests (96-h) with juvenile fish and four early life stage tests with embryos and larvae were completed with rainbow trout (<i>Salmo gairdneri</i>). Ninety-six-hour flow-through LC ₅₀ values for juveniles ranged from 8.1 to 10.9 mg/L nickel. Two early life stage tests were started with newly fertilized eggs, one test was started with eyed eggs and one test was started with pre-swim-up larval fish. No observed effect levels (NOEL), the highest nickel concentration without statistically significant adverse effects, for the two tests with newly fertilized eggs were less than 35 and 35 ug/L. The NOEL for the tests starting with eyed eggs and larval fish was 134 ug/L nickel. Early life stages were most sensitive when newly fertilized eggs were initially exposed, followed in sensitivity by eyed eggs, larval fish and juvenile fish.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

SENSITIVITY OF RAINBOW TROUT EARLY LIFE STAGES TO NICKEL CHLORIDE

ALAN V. NEBEKER,* CAROL SAVONEN and DONALD G. STEVENS

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory,
Corvallis, Oregon 97333

(Received 12 March 1984; Accepted 12 September 1984)

Abstract—Four acute tests (96-h) with juvenile fish and four early life stage tests with embryos and larvae were completed with rainbow trout (*Salmo gairdneri*). Ninety-six-hour flow-through LC50 values for juveniles ranged from 8.1 to 10.9 mg/L nickel. Two early life stage tests were started with newly fertilized eggs, one test was started with eyed eggs and one test was started with pre-swim-up larval fish. No observed effect levels (NOEL), the highest nickel concentration without statistically significant adverse effects, for the two tests with newly fertilized eggs were less than 35 and 35 $\mu\text{g/L}$. The NOEL for the tests starting with eyed eggs and larval fish was 134 $\mu\text{g/L}$ nickel. Early life stages were most sensitive when newly fertilized eggs were initially exposed, followed in sensitivity by eyed eggs, larval fish and juvenile fish.

Keywords—Rainbow trout *Salmo gairdneri* Nickel Acute toxicity Chronic toxicity
Early life stage tests

INTRODUCTION

This study of the effect of nickel on the early life stages of rainbow trout (*Salmo gairdneri*) was conducted to (a) provide nickel toxicity data for juvenile and early life stages of rainbow trout for the U.S. Environmental Protection Agency (EPA) Water Quality Criteria document for nickel [1]; (b) determine 96-h LC50 values for juvenile fish; (c) determine relative subacute sensitivity of early life stages and juveniles; and (d) determine if early life stage tests started with newly fertilized eggs are more sensitive than tests started with eyed eggs or larval fish.

Nickel has been shown to be moderately toxic to fish and aquatic invertebrates when compared with other metals [1], but only one 96-h LC50 (35.5 mg/L) has been calculated [2] for rainbow trout. Other fish species—goldfish, guppies and bluegills—had 96-h LC50s of 9.8, 4.4 and 5.2 mg/L, respectively [3]. Fathead minnow LC50s ranged from 25 to 32 mg/L [4]. A 48-h LC50 for *Daphnia magna* of 510 $\mu\text{g/L}$ has been calculated by Biesinger and Christensen [5], illustrating that organism's relative sensitivity compared with that of fish.

One early life stage study with salmonids and nickel was reported by Birge et al. [6], who calcu-

lated a 28-d LC50 of 50 $\mu\text{g/L}$ for rainbow trout embryos. Biesinger and Christensen [5] completed chronic 3-week studies with *D. magna* and calculated a 16% reproductive impairment concentration of 30 $\mu\text{g/L}$. Nebeker et al. [7] determined a no observed effect level (NOEL) of 66 $\mu\text{g/L}$ nickel for the caddisfly *Clistoronia magnifica*. Pickering [4] reported chronic "safe level" concentrations for fathead minnows of 527 $\mu\text{g/L}$ nickel in hard water and 109 $\mu\text{g/L}$ in soft water. More chronic data are needed to characterize the toxicity of nickel; this study expands that database.

METHODS AND MATERIALS

Test organism

Rainbow trout were used for all testing. Juveniles reared at our facility were used for acute testing. Two age groups, 3 months and 12 months old, from two year classes, were used to compare the effects of fish age on 96-h LC50 values. Newly fertilized eggs (less than 4 h post-fertilization), eyed eggs, and pre-swim-up larval fish [at swim-up fish have absorbed their yolk sac and they swim up off the stream (test container) bottom and begin to feed] were used for the four early life stage tests. All eggs were obtained from the Oregon Department of Fish and Wildlife Oakridge Hatchery. Gametes from three females and two males were pooled to obtain fertilized eggs, which

*To whom correspondence may be addressed.

were water-hardened for 1 h at the hatchery before transport to the laboratory. Swim-up fish were initially fed live, newly hatched brine shrimp; after the first week of feeding they were switched to Oregon Moist Pellet starter mash frozen fish food. Both diets were fed *ad libitum*.

Water

Water for all testing was obtained from wells and aerated to saturation prior to use. Dissolved oxygen was maintained near saturation (8.3–10.2 mg/L). Alkalinity, hardness and pH during acute juvenile testing were 27 to 30, 27 to 39 and 7.0 to 7.1 mg/L, respectively, and were monitored once during acute and weekly during chronic testing using procedures described by the American Public Health Association [8]. Initial water temperature during the chronic embryo exposures was set at 9.5°C (hatchery water temperature) and gradually raised to 12°C over a 3-d period. Water temperature during acute and chronic tests was maintained at 12 to 14°C.

Toxicant

Reagent-grade nickel chloride was used for all tests. Stock solutions were prepared by dissolving the nickel salt in acidified (0.1 ml HNO₃/L) well water held in a 400-liter polyethylene stock tank. The solution was pumped from the stock tank to the diluter. Water samples for total aqueous nickel analysis were taken from each test concentration daily during acute tests and at least weekly during the chronic tests, and were analyzed on a Perkin-Elmer 305B atomic absorption spectrophotometer with the direct aspiration (flame) procedure [8,9].

Exposure systems

Two continuous-flow diluter systems [10], capable of delivering five (acute tests) or 10 (chronic tests) concentrations of nickel to test containers, were used. The nickel stock solution was added to the incoming dilution water in the first chamber of the diluter with a pump set at a constant delivery rate of 37 ml/min. Acute tests with juvenile fish were conducted in 19-liter aquaria with a water flow to each aquarium of 350 ml/min. Early life stage tests were conducted in oval stainless steel troughs, 19-liter aquaria and 20 × 25 cm stainless steel screen cages in 100-liter aquaria. Fish from newly fertilized eggs were hatched in the oval troughs and then transferred to the 19-liter aquaria. The eyed eggs were hatched and fish retained in the oval troughs for the duration of the test. For the exposures starting with

pre-swim-up juveniles, fish were placed in the stainless steel wire cages suspended in the 100-liter aquaria. A 14-h light:10-h dark photoperiod was maintained for all exposures using wide-spectrum fluorescent lamps. The light intensity was near 1.1 lux at the water surface during most of the exposure, but was held near darkness during the sensitive embryonic stages.

Acute tests

Four acute tests were conducted (Table 1), two with 3-month-old fish and two with 12-month-old fish. The methods used for the 96-h flow-through exposures were basically those presented in "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" [11]. Five nickel concentrations plus a control were used for all tests, with a 50% dilution ratio between concentrations. Groups of 10 fish were randomly assigned to each aquarium (10 fish were also weighed and measured before test start), with two aquaria per concentration; dead fish were counted and removed daily. Fish were not fed during the acute exposure period. A 96-h LC50 value was calculated using the Trimmed Spearman-Kärber method [12] after combining mortality data from replicate aquaria.

Early life stage tests

Four tests were conducted: tests 1 and 2 starting with newly fertilized eggs 4-h post-fertilization, test 3 starting with eyed eggs 25 d postfertilization, and test 4 starting with 5-d post-hatch pre-swim-up larvae. The eyed eggs came from the same batch of eggs used for test 2 and were placed in the test water 4 d before hatch. The pre-swim-up larvae were also from the batch of eggs used in test 2, and were transferred to nickel-containing test water 1 week before swim-up. For tests 1 and 2, equal volumes (17 ml) of fertilized eggs were placed in the recirculating troughs, giving approximately 100 eggs per container. In test 3, 40 eyed eggs were counted and placed in each test container. In test 4, groups of 20 pre-swim-up larvae were randomly (5 larvae at a time) placed in each of the screen cages hung in the test water. Only four concentrations were used in test 4 because of space limitations. Unfertilized eggs and eggs containing dead embryos were removed daily and placed in Stockard's solution [13] for later examination. Unfertilized eggs were subtracted from egg counts for determining percent hatch and survival. The final weight of the fish included the remnants of the yolk sac in those fish that exhibited delayed

Table 1. Acute 96-h LC50 values for rainbow trout exposed to nickel chloride

Acute test no.	96-h LC50 (mg/L) ^a [confidence limits]	Fish age (mo)	Mean fish wt. (g)	Mean fish length (mm)	Mean temp. (°C)	Measured total nickel conc. (mg/L $\pm$ SD)	No. of fish dead (n = 20)
1	10.0 [8.8-11.4]	3	0.37	36	13.0	11.3 $\pm$ 0.1	13
						7.2 $\pm$ 0.1	2
						4.5 $\pm$ 0.1	0
						2.9 $\pm$ 0.1	0
						1.9 $\pm$ 0.1	0
						Control	0
2	10.9 [9.5-12.4]	3	0.58	40	13.2	19.4 $\pm$ 3.0	20
						12.5 $\pm$ 1.1	12
						6.3 $\pm$ 1.0	0
						4.5 $\pm$ 0.7	1
						3.3 $\pm$ 0.4	0
						Control	0
3	8.9 [7.5-10.6]	12	16.4	119	13.3	16.8 $\pm$ 0.3	19
						10.0 $\pm$ 0.2	11
						6.1 $\pm$ 0.2	3
						4.0 $\pm$ 0.1	3
						3.6 $\pm$ 0.1	1
						Control	0
4	8.1 [6.7-9.7]	12	15.4	116	14.0	12.2 $\pm$ 0.4	18
						7.7 $\pm$ 0.4	6
						5.1 $\pm$ 0.6	4
						2.9 $\pm$ 0.2	3
						2.2 $\pm$ 0.2	0
						Control	2

^aTrimmed Spearman-Kärber analysis.

growth at higher concentrations. Where high numbers of fish survived, only every second or third fish sampled was weighed. Dunnett's multiple comparison and chi-square analyses were used to determine significant differences ($\alpha = 0.05$) between treatment groups and controls, and to establish the NOEL (the highest nickel concentration in which growth was not significantly less than in controls).

RESULTS

Acute tests

The results of the four acute tests (Table 1) were consistent, with 96-h LC50 values ranging from 8.1 to 10.9 mg/L nickel. The older fish appeared to be somewhat more sensitive, but the confidence limits of both age groups overlapped, indicating that the LC50 values were not significantly different.

Early life stage tests

Test 1—newly fertilized eggs. No embryos survived to hatch at or above 1,680 μ g/L nickel

(Table 2). Many died during early embryonic development but these were not all detected until they failed to hatch. At 1,100 μ g/L, 3% hatched and 1% survived until the end of the test. Significant mortality occurred at and above 134 μ g/L, with significant effects ($\alpha = 0.05$) on growth (weight and length) at 35 μ g/L nickel, the lowest concentration tested. Fifty percent swim-up occurred 14 d after hatching in the controls. Swim-up was delayed to 16 d after hatching at 700 μ g/L and to 18 d at 1,100 μ g/L nickel. The NOEL was less than 35 μ g/L, with growth being a more sensitive parameter than survival.

Test 2—newly fertilized eggs. No embryos survived to hatch at or above 700 μ g/L nickel (Table 3). Five percent hatched at 431 μ g/L and 4% survived to the end of the test. Significant mortality occurred at and above 134 μ g/L, with significant effects on growth (weight) at 62 μ g/L. However, the impact of disease, both in the egg and in the developing larvae, makes these growth endpoints somewhat questionable. Fifty percent swim-up occurred 12 d after hatching in the con-

Table 2. Test 1—Rainbow trout early life stage test with nickel, starting with newly fertilized eggs^a

Mean ($\pm$ SD) measured total nickel conc. (μ g/L)	No. of fertilized eggs	Embryo survival (total no. of fish hatched)		Fish survival from hatch to end of test		Total survival from egg to end of test		Final mean fork length (mm)	Final mean wet weight (g)
		n	%	n	%	n	%		
3,730 $\pm$ 313	102	0	0	—	—	0	0 ^b	—	—
2,520 $\pm$ 203	103	0	0	—	—	0	0 ^b	—	—
1,680 $\pm$ 138	104	0	0	—	—	0	0 ^b	—	—
1,100 $\pm$ 109	103	3	3	1	3	1	1 ^b	33 ^b	0.34 ^b
700 $\pm$ 78	112	9	8	9	100	9	8 ^b	41 ^b	0.77 ^b
431 $\pm$ 80	106	34	32	25	74	25	24 ^b	41 ^b	0.74 ^b
238 $\pm$ 31	101	60	59	43	72	43	43 ^b	44 ^b	0.86 ^b
134 $\pm$ 19	105	90	86	57	63	57	54 ^b	44 ^b	0.83 ^b
62 $\pm$ 11	101	96	95	81	84	81	80	42 ^b	0.74 ^b
35 $\pm$ 14	103	94	91	86	91	86	83	43 ^b	0.84 ^b
Control	92	85	92	76	89	76	83	46	0.97

^aExposure began 4 h after fertilization.^bSignificantly different from control ($\alpha = 0.05$).Table 3. Test 2—Rainbow trout early life stage test with nickel, starting with newly fertilized eggs^a

Mean ($\pm$ SD) measured total nickel conc. (μ g/L)	No. of fertilized eggs	Embryo survival (total no. of fish hatched)		Percent ^b disease loss	Survival from hatch to end of test		Total survival from egg to end of test		Final mean fork length (mm)	Final mean wet weight (g)
		n	%		n	%	n	%		
3,730 $\pm$ 313	115	0	0	—	—	—	0	0 ^c	—	—
2,520 $\pm$ 203	113	0	0	—	—	—	0	0 ^c	—	—
1,680 $\pm$ 138	103	0	0	—	—	—	0	0 ^c	—	—
1,100 $\pm$ 109	97	0	0	—	—	—	0	0 ^c	—	—
700 $\pm$ 78	105	0	0	—	—	—	0	0 ^c	—	—
431 $\pm$ 80	114	6	5	0	5	83	5	4 ^c	38 ^c	0.61 ^c
238 $\pm$ 31	115	51	44	1	22	43	22	19 ^c	41	0.80
134 $\pm$ 19	111	72	65	5	26	36	26	23 ^c	42	0.84
62 $\pm$ 11	113	75	66	15	62	83	62	55	40	0.62 ^c
35 $\pm$ 14	112	75	67	9	49	65	49	44	42	0.76
Control	105	60	57	11	52	87	52	50	40	0.76

^aExposure began 4 h after fertilization.^bLarvae were lost to air-bladder fungus disease in addition to nickel toxicity.^cSignificantly different from control ($\alpha = 0.05$).

trols. Swim-up was delayed to 14 d after hatch at 431 and 238 μ g/L nickel. The NOEL was 35 μ g/L.

Test 3—eyed eggs. Twenty-eight percent of the fish survived at the highest test concentration—3,730 μ g/L (Table 4). Significant mortality occurred at and above 1,680 μ g/L nickel, with significant effects on growth (weight) at 431 μ g/L. Fifty percent swim-up occurred 13 d after hatching in the controls. Swim-up was delayed to 15 d

after hatching at 1,680 μ g/L, and to 20 d at 2,520 and 3,730 μ g/L nickel. The NOEL was 134 μ g/L.

Test 4—pre-swim-up larval fish. Fish were exposed to only four nickel concentrations: 3,730, 1,680, 700 and 134 μ g/L (Table 5). Five percent survived at the highest test concentration—3,730 μ g/L nickel. Significant mortality occurred at 1,680 μ g/L nickel, with significant effects on growth (weight and length) at 700 μ g/L. The

Table 4. Test 3—Rainbow trout early life stage test with nickel, starting with eyed eggs^a

Mean ($\pm$ SD) measured total nickel conc. (μ g/L)	No. of eyed eggs at test start	Embryo survival (total no. of fish hatched)		Fish survival from hatch to end of test		Total survival from egg to end of test		Final mean fork length (mm)	Final mean wet weight (g)
		n	%	n	%	n	%		
3,730 $\pm$ 313	40	35	88	11	31	11	28 ^b	28 ^b	0.16 ^b
2,520 $\pm$ 203	40	40	100	28	70	28	70 ^b	30 ^b	0.23 ^b
1,680 $\pm$ 138	40	39	98	28	72	28	70 ^b	32 ^b	0.33 ^b
1,100 $\pm$ 109	40	39	98	35	90	35	88	34 ^b	0.38 ^b
700 $\pm$ 78	40	40	100	36	90	36	90	35 ^b	0.41 ^b
431 $\pm$ 80	40	40	100	35	88	35	88	37	0.46 ^b
134 $\pm$ 19	39	38	97	31	82	31	79	38	0.56
35 $\pm$ 14	39	39	100	36	92	36	92	39	0.54
Control	40	40	100	39	98	39	98	38	0.55

^aExposure began 25 d after fertilization.^bSignificantly different from control ($\alpha = 0.05$).Table 5. Test 4—Rainbow trout early life stage test with nickel, starting with larval fish^a

Mean ($\pm$ SD) measured total nickel conc. (μ g/L)	No. of fish at test start	Survival at end of test		Final mean fork length (mm)	Final mean wet weight (g)
		n	%		
3,730 $\pm$ 313	20	1	5 ^b	33 ^b	0.37 ^b
1,680 $\pm$ 138	20	8	40 ^b	35 ^b	0.39 ^b
700 $\pm$ 78	20	18	90	40 ^b	0.62 ^b
134 $\pm$ 19	20	18	90	44	0.92
Control	20	19	95	45	1.02

^aExposure began with 5-d post-hatch fish, 1 week before swim-up.^bSignificantly different from control ($\alpha = 0.05$).

NOEL was 134 μ g/L nickel (Table 6). A 38-d LC50 value of 1.4 mg/L (confidence interval = 1.0–1.9 mg/L) was calculated for comparison with the 96-h values. There were no effects of nickel on swim-up of the fish at any concentration.

DISCUSSION

The 96-h LC50 values for juvenile rainbow trout exposed to nickel were similar to those found by Pickering and Henderson [3] for other fish species. The LC50 values for 3-month-old and 12-month-old fish were similar (Table 1), indicating that the age of juvenile fish probably will not significantly affect 96-h LC50 values. Longer exposures will reduce the LC50 values, as can be

seen by the mortality of pre-swim-up fish exposed in this study (38-d LC50 of 1.4 mg/L). The acute to chronic ratio for nickel, using chronic values of 35 and 134 μ g/L, was between 0.004 and 0.014.

Mortality occurred at much lower nickel concentrations in the early life stage tests than in the 96-h acute tests with juveniles. The tests begun with newly fertilized eggs resulted in lower NOEL values (less than 35 and 35 μ g/L) with nickel than did tests started with eyed eggs (134 μ g/L) or pre-swim-up larvae (134 μ g/L). This could be due to two factors; a greater sensitivity of the younger pre-eyed embryos to nickel, or longer exposure of the embryos and hatched fish to nickel in the test with newly fertilized eggs (Table 6). Three additional weeks of exposure are required for newly fertilized eggs, as compared with eyed eggs, to reach comparable terminal sizes. The test starting with pre-swim-up fish had the same NOEL as the eyed-egg test; however, no fish in test 4 were exposed at 431 μ g/L nickel (Tables 4 and 5), so we do not know if they would be affected at levels between 134 and 700 μ g/L.

The concentrations of nickel that did not cause any significant impairment of survival or growth (NOEL) in this study (35 μ g/L), starting with newly fertilized eggs, are similar to the nickel avoidance threshold value of 23.9 μ g/L found by Giattina et al. [14] for rainbow trout. They found that the avoidance threshold value of 23.9 μ g/L, at a hardness of 21 mg/L as CaCO₃, was close to the NOEL of 29 μ g/L, calculated using the U.S. EPA [1] criterion for nickel. Our values of less

Table 6. No observed effect levels (NOEL) and water quality values for rainbow trout early life stage tests with nickel^a

Early life stage test no.	Rainbow trout test started with:	Test duration (d)	NOEL ^b ($\mu\text{g/L}$)	Mean water temp. ($^{\circ}\text{C}$)	Median pH	Mean $\pm$ SD total alkalinity (mg/L)	Mean $\pm$ SD total hardness (mg/L)
1	Newly fertilized eggs	85	<35	12.1	7.0	45 $\pm$ 8	53 $\pm$ 10
2	Newly fertilized eggs	75	35	12.4	7.1	45 $\pm$ 6	52 $\pm$ 6
3	Eyed eggs ^c	52	134	12.5	7.1	39 $\pm$ 11	49 $\pm$ 7
4	Pre-swim-up larvae ^d	38	134	12.7	7.2	43 $\pm$ 9	50 $\pm$ 11

^aDissolved oxygen was at or near saturation at all times.

^bNOEL, the highest test concentration in which the effects were not significantly different from the control ($\alpha = 0.05$).

^c25 d after fertilization (4 d before hatching).

^d5 d after hatching (1 week before swim-up).

than 35 and 35 $\mu\text{g/L}$ nickel were also close to the U.S. EPA estimated NOEL for nickel.

Previous studies dealing with the relative sensitivity of different life stages of fishes, none with nickel but with other heavy metals [15,16], have also shown that the early stages are sensitive and need to be protected to maintain the species. Often the larval fish are more sensitive than the embryos because the toxic metal does not penetrate the protective egg shell or chorion [17]. Eaton et al. [18] showed that larvae or juveniles of seven fish species were more sensitive than were embryos. However, Spehar [19] showed that reproduction was a more sensitive response to cadmium with flagfish than was embryo or larval survival.

Acknowledgments—We wish to thank W. Miller and A. Purcell for assistance with nickel analyses, J. Oshiro and S. Onjukka for aid with data analysis, and the Oregon Department of Fish and Wildlife for the rainbow trout eggs.

REFERENCES

1. U.S. Environmental Protection Agency. 1980. Ambient water quality for nickel. EPA-440/5-80-060. U.S. EPA, Washington, DC.
2. Hale, J.G. 1977. Toxicity of metal mining wastes. *Bull. Environ. Contam. Toxicol.* 17:66.
3. Pickering, Q.H. and C. Henderson. 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. *Air Water Pollut. Int. J.* 10:453-463.
4. Pickering, Q.H. 1974. Chronic toxicity of nickel to the fathead minnow. *J. Water Pollut. Control Fed.* 46:760-765.
5. Biesinger, K.E. and G.M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. *J. Fish. Res. Board Can.* 29:1691-1700.
6. Birge, W.J., J.E. Hudson, J.A. Black and A.G. Westerman. 1978. Embryo-larval bioassays on inorganic coal elements and in situ biomonitoring of coal-waste effluents. In D. E. Samuel et al., eds., *Surface Mining and Fish/Wildlife Needs in the Eastern United States*. FWS/OBS-78/81 (as cited in U.S. EPA, 1980).
7. Nebeker, A.V., C. Savonen, R.J. Baker and J.K. McCrady. 1984. Effects of copper, nickel, and zinc on the life cycle of the caddisfly *Clistoronia magnifica* (Limnephilidae). *Environ. Toxicol. Chem.* 3:645-649.
8. American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. American Public Health Association, Washington, DC.
9. U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. U.S. EPA, Cincinnati, OH.
10. Nebeker, A.V., P. McKinney and M.A. Cairns. 1983. Acute and chronic effects of diflubenzuron (Dimilin) on freshwater fish and invertebrates. *Environ. Toxicol. Chem.* 2:329-336.
11. American Society for Testing and Materials. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-80. American Society for Testing and Materials, Philadelphia, PA.
12. Hamilton, M.A., R.C. Russo and R.V. Thurston. 1977. Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11:714-719. Correction 12:417 (1978).
13. Rugh, R. 1952. *Experimental Embryology*. Burgess Publishing Company, Minneapolis, MN.

14. Giattina, J.D., R.R. Garton and D.G. Stevens. 1982. Avoidance of copper and nickel by rainbow trout as monitored by a computer-based data acquisition system. *Trans. Am. Fish. Soc.* 111:491-504.
15. Sinley, J.R., J.P. Goettl, Jr., and P.H. Davies. 1974. The effects of zinc on rainbow trout in hard and soft water. *Bull. Environ. Contam. Toxicol.* 12:793-821.
16. Nebeker, A.V., C.K. McAuliffe, R. Mshar, and D.G. Stevens. 1983. Toxicity of silver to steelhead and rainbow trout, fathead minnows, and *Daphnia magna*. *Environ. Toxicol. Chem.* 2:95-104.
17. Stevens, D.G. and G.A. Chapman. 1984. Toxicity of trivalent chromium to early life stages of steelhead trout. *Environ. Toxicol. Chem.* 3:125-133.
18. Eaton, J.G., J.M. McKim and G.W. Holcombe. 1978. Metal toxicity to embryos and larvae of seven freshwater fish species. I. Cadmium. *Bull. Environ. Contam. Toxicol.* 19:95-103.
19. Spehar, R.L. 1976. Cadmium and zinc toxicity to flagfish. *J. Fish. Res. Board Can.* 33:1939-1945.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.